Reply to the comment by C.Camacho

In the recent Letter [1] we reported the results of simulations concerning chain length dependence of protein folding time. We argued that first order phase transition scenario where nucleus size does not depend on chain length and the role of surface energy is played by loop entropy (which depends logarithmically on chain length) may explain the observed power law dependence. In the comment [2] Camacho questions this interpretation by arguing that such a first order-like scenario yields folding time which is exponential in $N!$.

It is well-known that free energy barrier at first order transition is determined by the nucleus size which depends on interplay of bulk and surface free energy and does not depend on system size but rather on deviation from transition temperature. For this reason *at the transition temperature* in infinite *homogeneous system* the relaxation time diverges, because the critical nucleus size diverges. However, below the transition point the relaxation time of a first order transition is finite and by no means it is exponential in factorial of system size. It was made very clear in [1] that folding kinetics for each sequence was studied at the conditions *of its fastest folding*, rather than at temperature of folding transition. In all cases the temperature of fastest folding for designed sequences was markedly below the thermodynamic transition temperature. Therefore the allusion in [2] to the scaling of relaxation time at the point of transition is not applicable to simulations reported in [1]. Moreover, conclusion about divergence of relaxation time at the transition point is directly applicable only to homogeneous nucleation: in heteropolymeric system, even at the point where free energy of the folded state is equal to that of unfolded, some fragments of structure are more stable in their folded state than other and they can still serve as finite size nuclei for transition even at temperature of thermodynamic folding transition. For this reason simulations do not reveal any singular behavior of folding time in a wide range of temperatures and stabilities.

The analysis presented in [3] predicts second-order folding transition, contradicting the results of numerous simulations and analytical theories [4] which all converge that folding

transition in 3 dimensional proteinlike heteropolymer is a first order one. First, we note that the second order folding transition is in dramatic disagreement with experiment which shows that folding transition in the majority of relatively small (up to about 200 aminoacids) proteins is first-order like (for finite systems), both in thermodynamics and in kinetics [5].

Second, we point out that the conclusion about "second order" transition is a consequence of a crucial uncontrolled assumption made in [3]. The loop closure entropy was assumed in [3] to depend linearly on chain length. This is equivalent to neglecting chain connectivity in a polymer model. In order to see this, consider two monomers, say number i and j in sequence which are in contact with each other. This closes the loop of $j-i-1$ monomers between them. If conformational statistics of this loop is Gaussian, the entropic cost to bring a monomer k, which belongs to the loop (i.e. $i<k<j$), in contact with monomers i and j is $d/2(\log(k-i)+\log(j-k)-\log(j-i))$, (where d is space dimension), i.e it depends on position k of the monomer in sequence. This feature of the polymer model gives rise to sequence specificity, which is a key requirement for a model to be protein-like. However, in the model presented in [3] the loop entropy cost of bringing monomer k in contact with i and j from the previous example is $\lambda((k-i)+(j-k)-(j-i))=0$, which means that sequence dependence does not at all exist in this model. This makes the model of Camacho equivalent to a system of disconnected monomers occupying volume $V\sim N$. For this reason the "transition temperature" in [3], goes to zero in thermodynamic limit, i.e. strictly speaking, there is no folding transition in this model, at all.

It was argued in [3] that neglect of chain connectivity is a mean-field (MF) approximation equivalent to the one made in theory of random heteropolymers (RHP) [6,7]. This assertion is incorrect. In fact chain connectivity plays a crucial role in MF theory of RHP discussed in [7]. The easiest way to see this is to note that the physics of RHP, as predicted by MF theory dramatically depends on space dimension (which enters the theory via loop entropy factors) with $d>2$ and $d<2$ cases belonging to different universality classes (ultrametric landscape for $d<2$ and thermodynamic equivalence to the Random Energy Model at $d>2$ [7]). It is also clear that the model of Camacho is equivalent to zero-dimensional RHP, which explains

its inconsistency with basic thermodynamic properties of 3-dimensional heteropolymers and proteins.

The earlier theories of RHP are mean field ones not because they are based on some arbitrary assumptions but because they neglect fluctuations of certain order parameters, such as replica overlap $Q_{\alpha\beta}$ or microphase separation $m(r)$. In fact the validity of the MF approximation (Ginsburg number) in RHP theory was analyzed, and fluctuational corrections (mainly in one-loop approximation) were calculated in [8]

In summary we note that quantitative coincidence between simulations and inconsistent theory may be only fortuitous.

A. Gutin V.Abkevich and E. Shakhnovich
Harvard University
Department of Chemistry and Chemical Biology
12 Oxford Street, Cambridge MA 02138

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